Dietary leucine requirement of older men and women is higher than current recommendations

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ABSTRACT

Background: Current national (34 mg · kg^{-1} · d^{-1}) and international (39 mg kg^{-1} · d^{-1}) recommendations for leucine in older adults are based on data from young adults. Evidence suggests that the leucine requirements of older adults are higher than those of young adults.

Objective: The objective of the current study was to directly determine the leucine requirements in healthy older adult male and female study participants aged >60 y.

Methods: Leucine requirement was determined using the indicator amino acid oxidation method (IAAO) with L-[1-^{13}C]phenylalanine as the indicator. Sixteen older adults ($n = 7$ male and $n = 9$ female participants) were randomly assigned to receive 3 to 7 leucine intakes from 20 to 120 mg · kg^{-1} · d^{-1}. The rate of release of $^{13}CO_2$ from L-[1-^{13}C]phenylalanine oxidation was measured, and breakpoint analysis was used to estimate the leucine requirement. The 95% CI was calculated using the parametric bootstrap method.

Results: The mean leucine requirement for male participants was 77.8 mg · kg^{-1} · d^{-1} (upper 95% CI: 81.0) and for female participants, it was 78.2 mg · kg^{-1} · d^{-1} (upper 95% CI: 82.0) with no sex effect based on body weight. The data were therefore combined to yield a mean of 78.5 mg · kg^{-1} d^{-1} (upper 95% CI: 81.0 mg · kg^{-1} · d^{-1}) for both sexes. On the basis of fat-free mass, the mean ± SEM leucine requirements were 115 ± 3.2 and 127 ± 2.4 mg · kg^{-1} · d^{-1} for male and female participants, respectively ($P < 0.005$).

Conclusions: The estimated leucine requirement of older adults is more than double the amount in current recommendations. These data suggest that leucine could be a limiting amino acid in the diet of older adults consuming the current RDA for protein and those consuming a plant-based diet. In view of the functional and structural role of leucine, especially its importance in muscle protein synthesis, current leucine recommendations of older adults should be revised. This trial was registered at clinicaltrials.gov as NCT03506126. *Am J Clin Nutr* 2021;113:410–419.

Keywords: leucine requirement, amino acid requirement, elderly, indicator amino acid oxidation, carbon oxidation method, stable isotope, indispensable amino acid

Introduction

Aging is associated with a progressive loss of skeletal muscle mass, strength, and function (1). Among the indispensable amino acids, leucine is known to regulate intracellular pathways associated with muscle protein synthesis (MPS) (2). Specifically, leucine is directly involved in the activation of the mammalian target of rapamycin (mTOR) pathway, which plays a central role in mediating mRNA translation for MPS (3, 4). In addition, leucine may improve the action of insulin, allowing for increased uptake of glucose and amino acids into cells and decreased protein breakdown (5, 6). However, this relationship is complex and not yet completely understood.

Differences in leucine metabolism have been observed in older adults compared with young adults; splanchnic retention of leucine in the elderly is twice that observed in young adults (7), and the stimulatory action of leucine on muscle protein synthetic capacity decreases with age (8, 9). Data from physiological studies show older adults require approximately twice as much dietary leucine as young adults to achieve similar increases in MPS (10–14). Furthermore, some of the benefits derived from a

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Abbreviation used: APE, atom percent excess; BCAA, branched-chain amino acid; CRC, Clinical Research Center; DAAO, direct amino acid oxidation; EAR, estimated average requirement; $F^{13}CO_2$, rate of appearance of $^{13}CO_2$ in breath; FM, fat mass; FFM, fat-free mass; HbA1c, glycated hemoglobin; IAAO, indicator amino acid oxidation; IFCC, International Federation of Clinical Chemistry; MPS, muscle protein synthesis; mTOR, mammalian target of rapamycin; REE, resting energy expenditure; THb, total hemoglobin; WHR, waist-to-hip ratio.

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higher protein intake in older adults (1.0 or 1.2 g · kg^{-1} · d^{-1}) are predicated on an increased need for leucine in that population (15, 16).

To our knowledge, indispensable amino acid requirements have been scarcely studied in older adults, primarily because prior to the introduction of the minimally invasive indicator amino acid oxidation (IAAO) method, former methods have been too impractical for routine application in vulnerable populations like the elderly (17, 18). The resulting paucity of data has led to the application of young adult requirement data to older adult populations (19, 20). Therefore, the current leucine estimated average requirement (EAR) and RDA are set at 34 and 42 mg · kg^{-1} · d^{-1}, respectively, for all adults >19 y old, including older adults (19). These recommendations do not take into consideration the effect of aging and may not be adequate to support the needs of older adults. The aim of the current study, therefore, was to use the IAAO method to more accurately determine the leucine requirement of older adults. We hypothesized that in older adults the leucine requirement would be double the current recommendations of 34 mg · kg^{-1} · d^{-1}, previously derived in young adult male subjects.

Methods

Subjects

Nineteen healthy free-living older adults aged >60 y (9 male and 10 female subjects) were initially screened. Two men did not meet the inclusion criteria, and after recruitment, 1 female subject became ill (non–study related illness) and had to withdraw. Therefore, 16 older adults (7 males and 9 females) completed the study; 8 subjects were studied at 7 intake levels of leucine, 6 subjects at 5 intake levels, 1 subject at 4 intake levels, and 1 subject at 3 intake levels for a total of 93 IAAO experiments, completed in random order; details are shown in **Figure 1**.

The study was conducted in the Clinical Research Center (CRC), The Hospital for Sick Children, Toronto, Canada, between May 2018 and August 2019. Subjects were excluded if they had a recent history of weight loss, chronic disease, or acute illness that could affect protein and amino acid metabolism, e.g., diabetes, cancer, liver or kidney disease, and HIV. Subjects were also excluded if they were allergic or unable to tolerate the adaptation or experimental day diets. Subjects with hypertension were not excluded if their blood pressure was well controlled for at least 2 mo and their antihypertensive medications were taken as prescribed by their physician. The Research Ethics Board at The Hospital for Sick Children approved all procedures. Informed written consent was obtained from all participating subjects. Subjects received financial compensation for their participation.

Experimental design

The study design was based on the minimally invasive IAAO protocol (21). Following a 12-h overnight fast, a prestudy assessment of each subject's height, weight, fat mass (FM), fat-free mass (FFM), resting energy expenditure (REE), and medical history was conducted prior to initiation of the study. REE was measured by continuous, open-circuit indirect calorimetry (Vmax Encore, Metabolic cart; VIASYS), and FFM and FM were measured using 4 skinfold measurements, as previously described (22, 23). Waist and hip circumference measurements were taken and the waist-to-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference. Waist circumference was measured at the midpoint between the lower ribs and the iliac crest and hip circumference was measured at the greatest circumference of the buttocks. A 10-ml blood sample was taken to measure fasting glucose, glycated hemoglobin (HbA1c), urea, and creatinine to assess kidney function and whether subjects had diabetes.

Each level of leucine intake (20, 30, 45, 55, 70, 80, 90, 105, or 120 mg · kg^{-1} · d^{-1}) was studied over a 3-d period (24): 2 adaptation days followed by an IAAO experimental day, in a repeated measures design. During the 2 adaptation days, each subject received a lactose-free milkshake maintenance diet (Scandishake; Scandipharm), consumed as 4 equal meals (22–24). The same protein (1.0 g protein · kg^{-1} · d^{-1}) and energy (1.7 × REE) (22, 23, 25) were used for each subject (26). Subjects were adapted to 1.0 g protein · kg^{-1} · d^{-1} to ensure adequate protein intake and to minimize the effect of variation in prior protein intake on amino acid oxidation (26). On the third day (i.e., the IAAO experimental day), after a 12-h overnight fast, subjects consumed hourly meals containing a randomly assigned test level of leucine: 20, 30, 45, 55, 70, 80, 90, 105, or 120 mg · kg^{-1} · d^{-1}. A minimum of 3 points fell above and below the predicted breakpoint for statistical validity (18). Each 3-d period was separated by 1 to 2 wk from the subsequent and/or the previous test. The primary outcome of interest was L-[1-^{13}C]phenylalanine ($F^{13}CO_2$) in response to leucine intake. The secondary outcome was phenylalanine flux, because unchanging flux in response to the test amino acid intake is a criterion of the IAAO method (27).

Study diets

On the adaptation days, subjects were not allowed to consume anything else except water, plus 1 cup of clear tea or coffee. Subjects were given a daily 50+ multivitamin-mineral supplement (Centrum: Wyeth Consumer Health Care) for the duration of the study.

On each IAAO experimental day, subjects arrived at the CRC, where they consumed 8 hourly isocaloric meals—each meal representing one-twelfth of the daily requirement. The diet consisted of a liquid formula made with protein-free powder (PFD1; Mead Johnson), orange-flavored drink crystals (Fresh Plus Drink Crystals; WT Lynch Foods Limited), grape seed oil, a crystalline amino acid mixture patterned after egg protein, and protein-free cookies (28). The nitrogen content of the diets was adjusted according to the level of leucine intake with L-serine to keep the diets isonitrogenous. Total protein was provided at 1.0 g · kg^{-1} · d^{-1}. Energy was provided at 1.5× REE (25). Subjects were allowed water ad libitum on IAAO experimental days.

Tracer protocol

We previously demonstrated that route of tracer administration has no effect on amino acid requirement (29); therefore, we used our minimally invasive oral tracer infusion protocol in the current

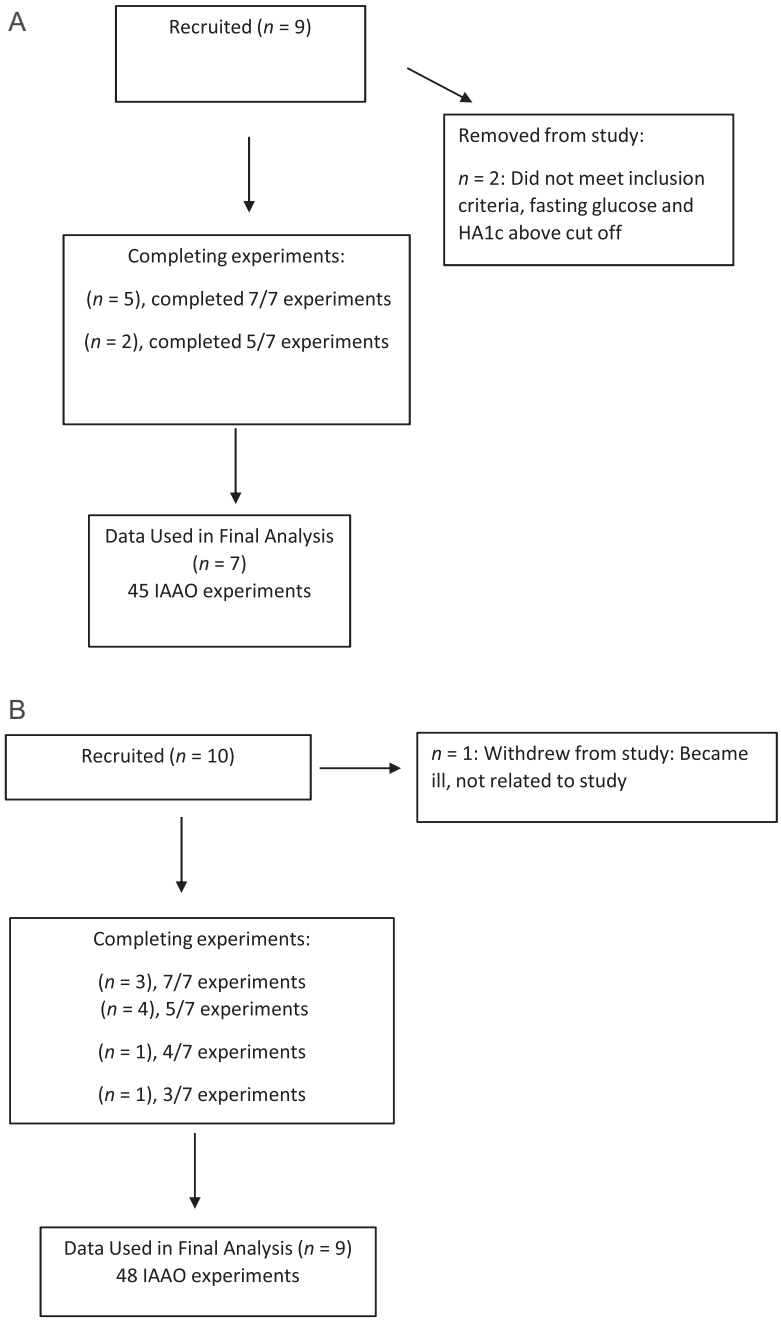

FIGURE 1 CONSORT flow chart of the study determining leucine requirement in (A) older males, and (B) older females, >60 y old, using the IAAO method. IAAO, indicator amino acid oxidation.

study. The oral tracer protocol on the IAAO experimental day began with the fifth meal. A priming dose of 0.176 mg · kg^{-1} of $NaH13CO_3$ [99 atom percent excess (APE); Cambridge Isotope Laboratories] and 0.66 mg · kg^{-1} of L-[1-^{13}C]phenylalanine (99 APE; Cambridge Isotope Laboratories) was given at the fifth meal. The fifth, sixth, seventh, and eighth meal contained the hourly dose of L-[1-^{13}C]phenylalanine (1.22 mg · kg^{-1} · h^{-1}). The amount of phenylalanine given as the tracer during the last 4 h of the study was subtracted from the total dietary phenylalanine intake, to ensure that total phenylalanine intake remained constant at 25 mg · kg^{-1} · d^{-1} (24). In addition, tyrosine was provided in excess at 40 mg · kg^{-1} · d^{-1} to ensure that the tracer was not delayed in the tyrosine pool (30).

Sample collection and analysis

Breath and urine samples were collected on all IAAO experimental days. Five baseline breath samples and 3 baseline urine samples were collected at 15- and 30-min intervals, respectively, before the tracer protocol began. Plateau breath and urine samples were collected in the same manner at isotopic steady state, which was reached 2.5 h after the start of the tracer

protocol. The collection and storage process has been previously described (22, 23). The volume of carbon dioxide production (VCO_2) in ml.min^{-1} was measured immediately after the fifth meal for a period of 20 min with continuous, open-circuit indirect calorimetry (Vmax Encore metabolic cart; VIASYS).

Expired $^{13}CO_2$ enrichment was measured using a continuous-flow isotope ratio mass spectrometer (CF-IRMS 20/20 isotope analyzer; PDZ Europa Ltd.) as previously described (22, 23). Enrichments were expressed as the APE compared with a reference standard of compressed carbon dioxide gas.

Urinary L-[1-^{13}C]phenylalanine enrichment was analyzed by an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems-MDS Sciex) in positive electrospray ionization mode as previously described (22, 23, 31). Isotopic enrichment was expressed as mole percent excess and calculated from peak area ratios at isotopic steady state at baseline and plateau (32). The CV between the $^{13}CO_2$ enrichment in the 5 breath samples at plateau was <5% and between the L-[1-^{13}C]phenylalanine in urine at plateau was <5%.

Blood was sent to the clinical chemistry department at the Hospital for Sick Children for analysis of glucose, urea, creatinine, and HbA1c. Serum glucose, urea, and creatinine concentrations were determined using calorimetric reaction with dye and enzymatic reactions with glucose oxidase, urease, and creatinine amidohydrolase, respectively. The concentrations of serum glucose, urea, and creatinine in 10-, 5.5-, and 6-μL samples were determined on an Ortho Vitros 4600 automated chemistry analyzer (Ortho-Clinical Diagnostics, Inc.), at 37°C at λ values of 540, 670, and 670, respectively. Precision and accuracy of the instrument were both <5%.

The HbA1c assay utilized an enzymatic method that specifically measures the N-terminal fructosyl dipeptides of the β-chain of HbA1c. In the pretreatment process, the erythrocytes were lysed and the hemoglobin transformed to methemoglobin by reaction with sodium nitrite. With the addition of 10-(carboxymethylaminocarbonyl)-3,7-bis(dimethylamino) phenothiazine sodium salt and protease (bacterial) to the sample, the glycosylated N-terminal dipeptide (fructosyl-VH) of the β-chain of hemoglobin was cleaved by the action of protease. The hemoglobin was then transformed to stable methemoglobin azide by the action of sodium azide and the concentration of the hemoglobin was determined by measuring absorbance at λ of 660. Addition of peroxidase (horseradish) and fructosyl-peptide-oxidase started a reaction, and the HbA1c concentration was measured by determining the resultant hydrogen peroxide. Total hemoglobin (THb) was determined by oxidation to stable methemoglobin azide by the action of sodium nitrite and sodium azide at a λ of 660. The final result was expressed as percent HbA1c and was automatically calculated by the system from the HbA1c/THb ratio as follows: mmol/mol HbA1c IFCC (International Federation of Clinical Chemistry): HbA1c (mmol/mol) = (HbA1c/THb) × 1000 %HbA1c DCCT (Diabetes Control and Complications Trial)/NGSP (National Glycohemoglobin Standardization Program): HbA1c (%) = IFCC × 0.09148 + 2.152.

Estimation of isotope kinetics

Isotopic steady states in the tracer enrichment at baseline and plateau were represented as the unchanging values of L-[1-^{13}C]phenylalanine in urine and $^{13}CO_2$ in breath. Phenylalanine flux (μmol · kg^{-1} · h^{-1}) was calculated from the dilution of orally administered L-[1-^{13}C]phenylalanine into the metabolic pool (at steady state) by using enrichment of L-[1-^{13}C]phenylalanine in urine (21, 29). The rate of release of $^{13}CO_2$ after the oxidation of ingested L-[1-^{13}C]phenylalanine ($F^{13}CO_2$ μmol · kg^{-1} · h^{-1}) was calculated according to the stochastic model of Matthews et al. (32), by using a factor of 0.82 to account for carbon dioxide retained in the body's bicarbonate pool (33).

Statistical analysis

The following statistical analyses were performed with R (R version 3.6.2) for Windows. Statistical analysis was performed on primary and derived variables, and data were expressed as means ± SDs. Significance was established at $P < 0.05$.

The mean leucine requirement was estimated by applying a biphasic linear mixed-effects model to the $F^{13}CO_2$ data (34). The test involves partitioning of the data above and below the predicted breakpoint with 2 separate linear mixed models: a downward (i.e., negative) slope before the breakpoint, and a flat (i.e., zero) slope above the breakpoint. The leucine requirement (EAR) was defined as the breakpoint—the point at which phenylalanine oxidation and $F^{13}CO_2$ data plateaus and is no longer influenced by increasing leucine intake (35). The 95% CIs were calculated using parametric bootstrap (36).

To test whether the breakpoints were different between males and females in the current study or between older adults in the current study and young adults in the study of Kurpad et al. (37), the overlap in the CI was calculated as previously described (38): $((\text{breakpoint}_1 - \text{breakpoint}_2) \pm 1.96\ [\sqrt{(SE_1{}^2 + SE_2{}^2)}]$. The null hypothesis was accepted if the interval contained zero.

The order of testing the leucine intakes was randomly assigned within subjects, with the level of leucine intake serving as the main treatment effect. The fixed effects of leucine intakes, sex, FFM, BMI, and WHR on phenylalanine flux were tested using a joint linear mixed-effect model with the subject as a random effect term using the R package nlme.

To investigate what predictors (FFM, BMI, and WHR) can explain the phenylalanine flux difference in sex, we set the aforementioned joint linear mixed model and used the fitted value to impute the counterfactual outcome for each observation pretending the corresponding subject had a different sex. We then calculated the difference using the original observation and the counterfactural outcome and fitted a joint linear mixed-effects model by treating the difference as the outcome; the FFM, BMI, and WHR as fixed effect terms; and the subject as a random effect term.

A *t*-test was used to assess for differences between male and female subject characteristics. The leucine requirement per kg FFM was derived from the mean leucine requirement per kg body weight divided by the FFM and differences between males and females assessed using a *t*-test.

Results

Subject characteristics

Sixteen healthy older males (aged 70.4 ± 2.2 y) and females (70.7 ± 1.7 y) completed the study (**Table 1**). Body mass indices were 27.2 ± 0.6 and 29.2 ± 1.3 kg.m^{-2} for males and females, respectively, and body weight was maintained by each subject

TABLE 1 Subject characteristics of healthy older males and females who completed participation in the study to determine leucine requirement in older adults[1]

Characteristics	Value	
	Older males ($n = 7$)	Older females ($n = 9$)
Age, y	70.4 ± 2.2	70.7 ± 1.7
Weight, kg	83.9 ± 4.5	76.0 ± 3.4
Height, cm	175 ± 3.5[b]	161 ± 2.1[a]
BMI, kg m^{-2}	27.2 ± 0.6	29.2 ± 1.3
WHR	0.95 ± 0.03[b]	0.88 ± 0.01[a]
FFM-SF,[2] kg	56.5 ± 2.4[b]	45.9 ± 1.9[a]
% Fat-SF[2]	32.3 ± 1.8[b]	38.6 ± 1.1[a]
REE,[3] kcal d^{-1}	1523 ± 50[b]	1353 ± 52[a]
Blood HbA1c, %	5.26 ± 0.12	5.22 ± 0.11
Fasting blood glucose, mmol L^{-1}	5.4 ± 0.06	4.93 ± 0.23
Fasting blood urea, mmol L^{-1}	6.84 ± 0.55	6.42 ± 0.28
Fasting blood creatinine, μmol L^{-1}	80.1 ± 3.5[b]	67.2 ± 3.9[a]

[1]All values are means ± SEMs. Values with different superscripts were significantly different, $P < 0.05$, determined by *t*-test. FFM, fat-free mass; HbA1c, glycated hemoglobin; REE, resting energy expenditure; SF, skinfold; WHR, waist-to-hip ratio.
[2]Determined by skin fold analysis.
[3]Determined by open-circuit indirect calorimetry.

throughout the entire 4- to 6-mo study period with a change of ±0.62 kg for males and females, which was not significant.

Body composition (FFM and percent fat) and REE were significantly different between the sexes ($P < 0.05$). Blood creatinine was also significantly different between the sexes ($P < 0.05$). All subjects had normal kidney function and none had diabetes (Table 1).

Phenylalanine flux

Phenylalanine flux was not affected ($P = 0.67$ and $P = 0.56$ for males and females, respectively) by leucine intake as required by the IAAO method. This finding provides evidence that the precursor pool of the indicator amino acid did not change with changes in leucine intake and suggests that changes in phenylalanine oxidation are inversely related to changes in whole-body protein synthesis. Phenylalanine flux values were 58.0 ± 4.12 and 81.4 ± 4.50 (mean ± SEM) for males and females, respectively ($P = 0.002$) (**Table 2**). Flux was significantly associated with WHR and sex ($P = 0.005$ and $P = 0.028$, respectively). FFM, BMI, and WHR explained 38.4% ($P = 0.004$), 20.2% ($P = 0.019$), and 9.5% ($P = 0.08$) of the difference in flux between males and females, $R^2 = 0.84$.

Leucine requirement estimated from L-[1-^{13}C]phenylalanine oxidation ($F^{13}CO_2$)

The rate of release of $^{13}CO_2$ from L-[1-^{13}C]phenylalanine oxidation ($F^{13}CO_2$) progressively decreased as leucine intake increased from 20 to 80 mg · kg^{-1} · d^{-1} in both older males and females. Further increases in leucine intake did not result in changes in $F^{13}CO_2$, suggesting that the requirement was met. Biphasic linear regression analysis of the $F^{13}CO_2$ data resulted in the identification of a breakpoint for the mean leucine requirement of 77.8 mg · kg^{-1} · d^{-1} ($R^2 = 0.449$, $P < 0.0001$) for males and 78.2 mg · kg^{-1} · d^{-1} ($R^2 = 0.468$, $P < 0.0001$) for females (**Figure 2**A). The safe population level estimated by determining the upper 95% CI of the breakpoint was at 81.0 mg · kg^{-1} · d^{-1} for males and 82.0 mg · kg^{-1} · d^{-1} for females.

TABLE 2 Effect of leucine intake on phenylalanine flux in older males and females who participated in the study to determine leucine requirement in older adults aged >60 y[1]

Leucine intake, mg · kg^{-1} · d^{-1}	Phenylalanine flux,[2] μmol · kg^{-1} · h^{-1}	
	Males	Females
20	64.6 ± 12.6	88.8 ± 16.3
30	46.4 ± 2.00	
40	58.6 ± 13.9	85.2 ± 12.6
55	55.8 ± 6.72	77.7 ± 11.5
70	65.1 ± 20.9	72.3 ± 6.80
85	49.7 ± 4.24	83.0 ± 13.1
95	61.0 ± 9.70	82.5 ± 13.8
105	45.5 ± 2.57	
120	71.1 ± 21.0	77.1 ± 4.42
Mean ± SEM	58.0 ± 4.12[a]	81.4 ± 4.50[b]

[1]All values are means ± SEMs, $n = 45$ and 48 for males and females, respectively. Joint linear mixed model values with different superscripts are significantly different.
[2]Leucine intake had no statistically significant effect on phenylalanine flux for both males and females, $P > 0.05$ by ANOVA.

Estimation of the overlap in the CI of the 2 estimates revealed an interval of zero; therefore, the null hypothesis of no difference was accepted. Since the leucine requirements of the older males and females were not significantly different on the basis of body weight, we therefore combined and reanalyzed the data to estimate a breakpoint and 95% CI for the entire group of males and females combined. Biphasic linear regression analysis of the $F^{13}CO_2$ data resulted in a breakpoint of 78.5 mg · kg^{-1} · d^{-1} ($R^2 = 0.456$, $P < 0.0001$) and upper 95% CI of 81.0 mg · kg^{-1} · d^{-1} for males and females combined (Figure 2B).

Leucine requirement based on FFM

Figure 3 presents the leucine requirement per kg FFM. The requirement was 115 ± 3.2 and 127.6 ± 2.4 (mean ± SEM) for males and females, respectively ($P = 0.005$). The requirement estimate per kg FFM ranged from 105.1–132 and 121.1–137.3 for males and females, respectively. The 95% CIs of the estimates were 109.4, 125.4 for males and 120.4, 133.7 for females.

Comparison of current leucine requirement estimates in older adults to young adult data

Estimation of the overlap in the 95% CI of the requirement estimate in the current study and the study of Kurpad et al. (37) did not include zero; therefore, the null hypothesis of no difference was rejected. The mean and 95% CI in the study of Kurpad et al. were 37.3 and 20, 50 mg · kg^{-1} · d^{-1}, respectively.

Discussion

This is the first study to our knowledge to directly determine the dietary leucine requirement of older adults aged >60 y. Using the minimally invasive IAAO method, we identified EARs

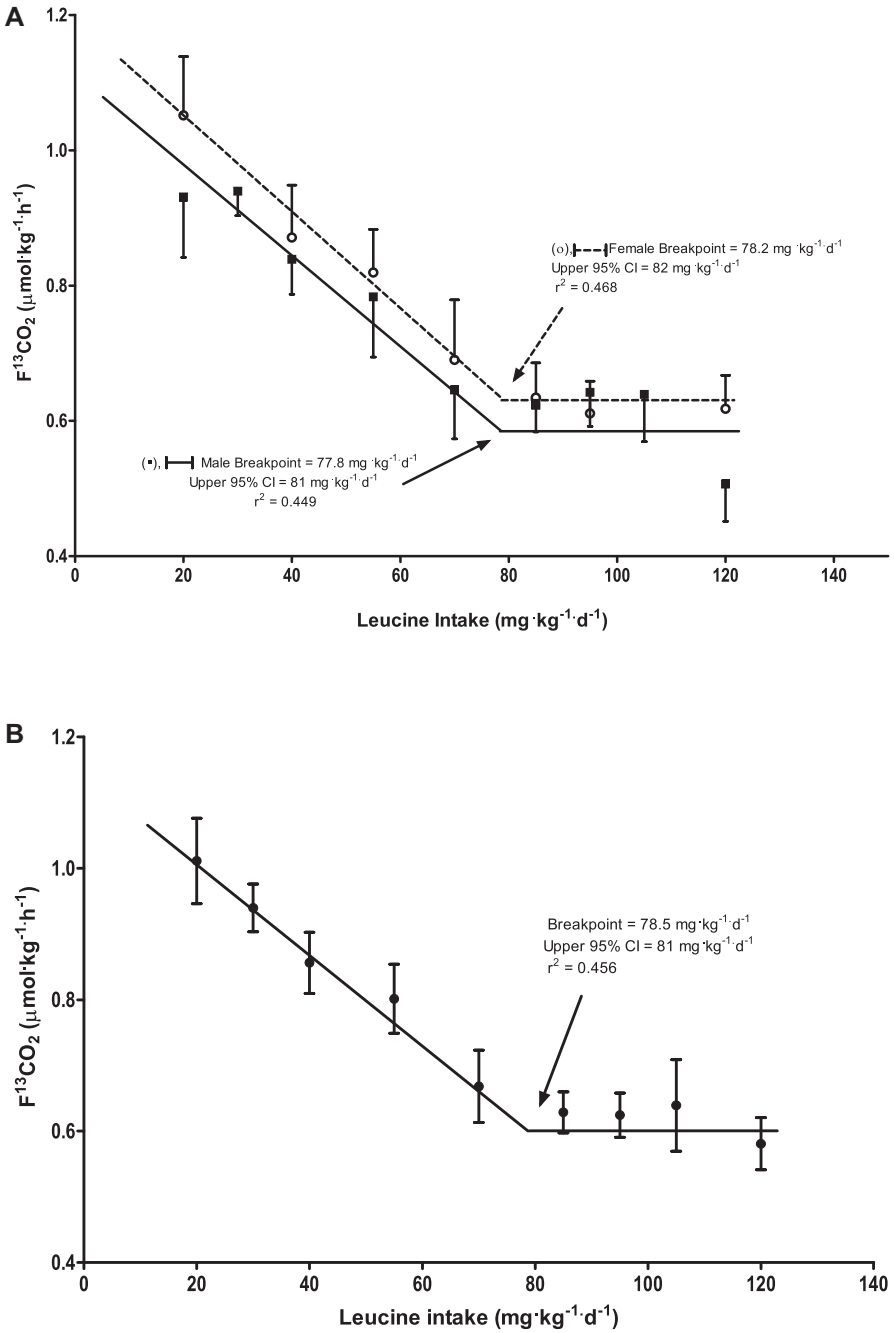

FIGURE 2 Effect of leucine intake on production of $^{13}CO_2$ from phenylalanine oxidation ($F^{13}CO_2$) by IAAO in older adults aged >60 y, $n = 16$ subjects, 93 IAAO studies. Biphasic linear regression analysis of the $F^{13}CO_2$ data identified a breakpoint of 77.8 mg · kg^{-1} · d^{-1}, R^2 0.45, $P < 0.0001$, for the males and 78.2 mg · kg^{-1} · d^{-1}, R^2 0.47, $P < 0.0001$, for the females (A), which represents the estimated mean leucine requirement. The 95% CIs of the estimates were 74.7, 81.0, and 74.5, 82.0 mg · kg^{-1} · d^{-1} for males and females, respectively. The breakpoint and the 95% CIs of the breakpoints for males and females combined (B) were determined to be 78.5 mg · kg^{-1} · d^{-1}, and 76.1, 81.0 mg · kg^{-1} · d^{-1}, R^2 0.456, $P < 0.0001$. IAAO, indicator amino acid oxidation.

and RDAs for dietary leucine of 77.8 and 81.0 and 78.2 and 82.0 mg · kg^{-1} · d^{-1} for males and females, respectively, with no difference in requirements between the sexes on a body weight basis. Therefore, we combined all data for a group EAR and RDA of 78.5 and 81.0 mg · kg^{-1} · d^{-1} for dietary leucine in older adults aged >60 y. Although no sex differences exist when expressed on the basis of body weight, the requirement was significantly different between males and females when expressed on the basis of FFM, with estimates for males and females of 115 ± 3.2 and 127.6 ± 2.4 mg · kg^{-1} · d^{-1}, respectively. Females had significantly lower FFM values than males, and sex dimorphisms in MPS have been described in the elderly (39, 40), with females having higher basal MPS rates and a greater resistance of MPS to exogenous protein intake. The availability of essential amino acids (41), particularly leucine (2, 15, 16), is an important regulator of MPS; hence, when a protein-deficient meal or

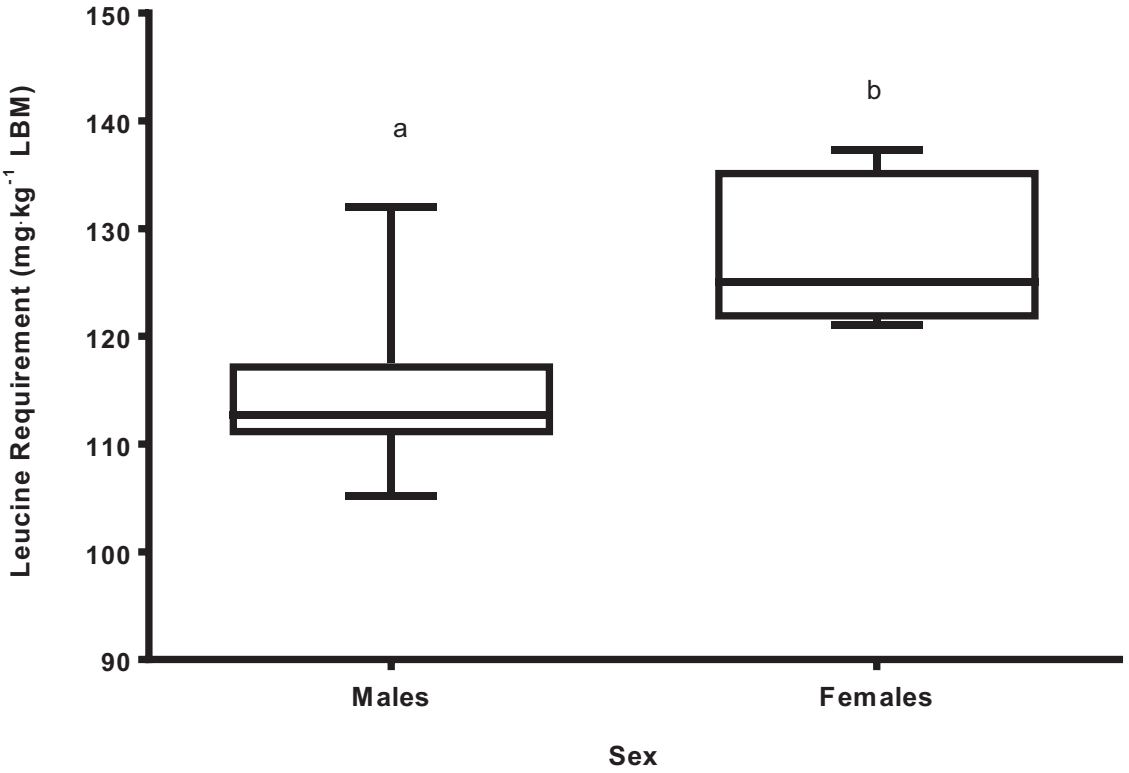

FIGURE 3 Leucine requirement per kg fat-free mass in older males and females who completed the study to determine leucine requirements in adults aged >60 y. Different superscripts show significant differences, $P = 0.005$, in leucine requirement per kg lean body mass (LBM) between males and females, compared by *t*-test.

inadequate leucine is consumed, protein synthesis is minimized (16). In addition, increased splanchnic uptake of leucine has been observed in the elderly and was associated with BMI (7). In the current study, the difference in BMI between males and females was not statistically significant, but at 27.2 ± 0.6 for males and 29.2 ± 1.3 for females is likely clinically significant and could have accounted for a higher splanchnic uptake of leucine in females, resulting in lower peripheral leucine availability and, hence, an increased requirement on the basis of FFM.

The mean leucine requirement is >2 times the current recommendation derived in young adults (19). A limitation of the current study is that we did not include a young adult control group. Nevertheless, we compared our data to the best comparable published data in young adults, which was done using carbon oxidation (37). Using the overlap of the 95% CIs of the breakpoints, we found that the interval did not contain zero. This result provides the evidence to suggest that the current estimate of the leucine requirement in older adults is higher than the leucine requirement in young adults. This is not a surprising finding. Earlier in vitro observations in rat muscle demonstrated resistance to leucine stimulation on MPS in older compared with young rats (8). In older humans, the blunted response of MPS to essential amino acids was reversed by leucine, but the amount of leucine was almost twice that required in young adults to stimulate similar rates of MPS (11). These observations have been extended to feeding trials in elderly humans, in which leucine coingestion with meals stimulated increased MPS in older males consuming a basal leucine intake of 63 to 100 mg · kg^{-1} · d^{-1} from a mixed protein diet (12). The same investigative group recently showed that leucine is the key stimulator of MPS in healthy elderly females (15). Interestingly, when the MPS response to leucine intake is compared across studies of older males and females (15, 39, 40, 42, 43), females exhibit stimulation of MPS, albeit to a lesser extent than seen in males, suggestive of a reduced responsiveness of MPS to stimulation by leucine. In the current study, although we did not find a difference in the leucine requirement between the sexes on the basis of body weight, when expressed on the basis of FFM, there was an 11% higher leucine requirement in females, which is statistically significant (Figure 3).

We previously reported that the protein requirement of elderly females (22) and males (23), approximating the same age as those in the current study, was comparable to that in young adult men (44). We are unable to find any evidence that the indispensable amino acid composition of whole-body protein changes with age (19). However, the mean leucine requirement and upper 95% CI estimate for leucine obtained in the current study can be provided by 1.0 to 1.2 g · kg^{-1} · d^{-1} of high-quality protein. This protein intake represents the mean and 95% CI estimated from our protein requirement studies in male and female adults aged >65 y (22, 23). This finding may suggest that the requirement for leucine is a key factor behind the requirement for protein in older adults. This idea is in support of recent data from Devries et al., who showed that leucine was the most important constituent amino acid in protein driving MPS in older females (15).

Leucine kinetics in young and elderly males was reported in an elegant paper (7) in which splanchnic retention of leucine was 50% in elderly compared with 23% in the young. The investigators' (7) initial approach was to use a [$^2H_3$]-leucine oral tracer and a [1-^{13}C]leucine intravenous tracer; however, when they switched the route of tracer administration, leucine oxidation was 73% in the first study and 173% when the [^{13}C] leucine was given orally. This contrasts markedly with the report of findings in young adults by Matthews et al. (45), in which <2% of nasogastric [1-^{13}C] leucine was oxidized on first pass. These authors estimated that 40% of the leucine tracer retained in the splanchnic bed was converted to α-ketoisocaproate and released to the systemic circulation and that 50% was incorporated into newly synthesized protein. It appears that in the elderly oral leucine is oxidized to a measurable extent in the splanchnic bed. We were not able to find a reason why. It may, however, be a contributing factor as to why elderly subjects have significantly greater dietary leucine requirements.

In the current study, we observed a greater phenylalanine flux in the older female than the older male participants (Table 2). Almost 60% of the difference in phenylalanine flux between males and females was explained by FFM and BMI. Females had a higher flux due to a lower urinary phenylalanine enrichment. This finding is supported somewhat by data from Volpi et al. (46), who demonstrated higher splanchnic phenylalanine uptake in older adults but, unfortunately, Volpi did not report on sex differences in the elderly. It is possible that, as in the findings of Boirie et al. (7), the effect of BMI in the current study was manifested by higher splanchnic extraction, contributing to the lower urinary enrichments and higher flux. This possibility needs to be investigated further. Like the positive correlation between splanchnic extraction and body fatness assessed by BMI and observed in Boirie's study (7), obese rats and humans have higher splanchnic extraction of dietary amino acids, enlarged livers, and more insulin resistance than nonobese subjects (47, 48). This may provide some explanation for our observation of higher phenylalanine flux in older females than males because, although not statistically significant, females in the current study had a higher BMI than males.

Assuming that mixed muscle protein contains 8.1 g leucine per 100 g of protein (49), our estimated average leucine requirements of 78.5 mg $\cdot$ kg$^{-1}\cdot$ d^{-1} and upper 95% CI of 81.0 mg $\cdot$ kg$^{-1}\cdot$ d^{-1} would require the elderly to consume between 1.0 and 1.2 g $\cdot$ kg$^{-1}\cdot$ d^{-1} of high-quality protein to meet their leucine requirement. Our data also raise the question of whether exclusively plant-based diets are feasible for older adults. From the standpoint of environmental sustainability, although plant foods may be considered advantageous compared with animal foods, this may not be true considering that older adults need far greater quantities of plant foods to meet their leucine requirement (50). Because of the relatively low protein content of plant foods and significantly higher requirement for leucine in older than in young adults, leucine can be considered a limiting amino acid in the diet of elderly individuals on a plant-based diet as well as in the diet of those getting <1.2 g $\cdot$ kg$^{-1}\cdot$d^{-1} high-quality protein. Ingesting a leucine supplement with each protein-containing meal may be an efficient and practical means of meeting the dietary leucine requirement in the elderly (12, 14), particularly in those dependent on a plant-based diet or those who habitually consume protein close to the current published RDA of 0.8 g $\cdot$ kg$^{-1}\cdot$ d^{-1}.

The IAAO method is widely accepted as a valid method for determining indispensable amino acid requirements. However, the method has received some criticism, one concern being that the continuous hourly meal protocol with free crystalline amino acids is not representative of the natural pattern of feeding involving 3 meals per d containing intact protein. Indeed, free crystalline amino acids are considered mostly bioavailable, whereas dietary proteins vary in their rate of absorption and bioavailability (51). However, it was previously demonstrated that animals grew at the same rate and the patterns of oxidation were the same whether free amino acids or protein bound amino acids were provided with frequent feeding. With feeding of 1 meal per d, amino acids were inefficiently used, whether provided as free or protein bound amino acids (52). Hence, based on the frequent feeding protocol used in the current study, we do not expect that the free amino acids provided in the mixed diet were used inefficiently.

Another concern that has been raised is that the short-term nature of the IAAO method may not lend enough support for a requirement estimate that is reflective of long-term health benefits. Admittedly, a longer-term trial would lend greater support for the higher leucine requirement in older adults, although such studies are difficult to conduct. However, the current IAAO estimate could serve as the basis for a longer-term feeding trial.

In conclusion, this is the first study to determine the leucine requirement of older adults using the IAAO method. The EAR and RDA of 78.5 and 81 mg $\cdot$ kg$^{-1}\cdot$ d^{-1} of dietary leucine for older adults is more than double the current recommendations set by the Institute of Medicine. Therefore, the current recommendations will need to be revised for older adults. Future longer-term studies based on functional markers of adequacy are needed to validate these results. Additionally, other indispensable amino acid requirements should be investigated in this population. It is important that older adults have specific nutritional recommendations aimed to support their health and functional well-being. The older adult population is considered the fastest growing demographic, and thus, it is imperative that such studies be conducted with a sense of urgency.

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Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

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